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DRUG SYNTHESIS

SYNTHESIS, PHYSICOCHEMICAL AND ANTICONVULSANT PROPERTIES OF NEW *N*-4-ARYLPIPERAZIN-1-YL AMIDES OF (2-AZA-1,3-DIOXOSPIRO [4.5] DEC-2-YL)-ACETIC ACID

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Abstract: As a continuation of our study on a number of 1,3-substituted pyrrolidine-2,5-diones, in this paper we report the synthesis, physicochemical and anticonvulsant properties of new derivatives of *N*-4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid. The amides [**II-X**] were prepared by condensation of the formerly obtained (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid [**I**] with the appropriately substituted 4-arylpiperazines in DMF, in the presence of the *N,N*-carbonyldiimidazole (CDIM) reagent at room temperature. The compounds were tested for their anticonvulsant activity in the maximum electroshock seizure (MES) and the metrazole seizure threshold (sc. Met) tests. Some of them were active in the sc. Met test. The structures of the new amides were confirmed by their elemental and spectral analyses.

Keywords: *N*-4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid, spirosuccinimides, pyrrolidine-2,5-diones, anticonvulsant activity

The currently available anticonvulsant drugs are effective in reducing the severity and number of seizures in less than 80% of the treated patients (1). Moreover, their usage is associated with side-effects ranging from cosmetic (gingival hyperplasia) to life--threatening (hepatotoxicity, megaloblastic anemia) (2, 3). Thus, the evolution of novel antiepileptic agents is of urgent necessity. It is well known that numerous derivatives with anticonvulsant activity do contain 5- or 6-membered heterocyclic rings, one or two carbonyl groups, as well as an aromatic system (4, 5). Among compounds investigated for their anticonvulsant activity, one of the structural features that play a significant role for their enhanced activity is just an amide fragment (6-8).

The study carried out by Scott at al. (9, 10) on a group of spiro[4.5] or spiro[4.4] carboxylic acids as cyclic analogues of valproic acid has demonstrated an anticonvulsant activity of these compounds. Further investigation of the group of spirosuccinimides (11) has revealed a key role of the cyclic system connected with an imide fragment through a spiro carbon atom, regarding the influence of compounds of that type on the anticonvulsant activity.

Following these findings, in the course of developing some new, potentially anticonvulsant compounds, our attention has been focused on a group of 3-substituted pyrrolidine-2,5-diones with various substituents at the nitrogen atom (12-15). Recently, we have shown that a great number of pyrrolidine--2,5-dione derivatives with a 4-arylpiperazine moiety at the N1 position, have exhibited a notable anticonvulsant activity (16), especially in the maximum electroshock (MES) test (e.g. *N*-{[4-(3-chlorophenyl)-piperazin-1-yl]-methyl}-3-(2-chloro-phenyl)-pyrrolidine-2,5-dione; ED_{50} = 14.20 mg/kg). On the contrary, 3-spirocycloalkyl analogues were effective in the sc. Met test (17).

In our previous study, we described the synthesis and physicochemical properties of *N*-phenyl and *N*-benzyl amides of 3-spirocycloalkylpyrrolidine--2,5-dione acetic and benzoic acids which, unfortunately, were devoid of any anticonvulsant activity (18). It is well known that the 4-arylpiperazine moiety plays an essential role as a pharmacophoric substituent, and is present in many compounds exhibiting a variety of pharmacological effects (19-20). These findings and the information given above have prompted us to carry out the synthesis of new amides of (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid in which we have replaced the phenyl- and benzyl-amines with a 4-arylpiperazine fragment having various substituents at the aryl ring (see Figure 1).

All the newly obtained compounds **II–X** were tested for their anticonvulsant activity through the Anticonvulsant Screening Program (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) in the maximal electroshock (MES), subcutaneous metrazole (sc. Met) and neurotoxicity (Tox) tests. The starting 1-carboxy-1-cyclohexane-

-acetic acid was synthesized by a method previously reported (21). The synthesis and physicochemical data of (2-aza-1,3-dioxo-spiro[4.5]dec-2-yl)-acetic acid [**I**] were described in our recent publication (18). The reaction of acid [**I**] with the appropriately substituted 4-arylpiperazines, in the presence of carbonyldiimidazole (CDIM) (22) in DMF, finally led to *N*-4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid [**II-X**].

^{1}H-NMR spectra of the synthesized compounds [**II--X**] were studied. They ^{1}H NMR spectra revealed a few characteristic chemical shifts of the investigated amides. The chemical shifts of the cyclo-

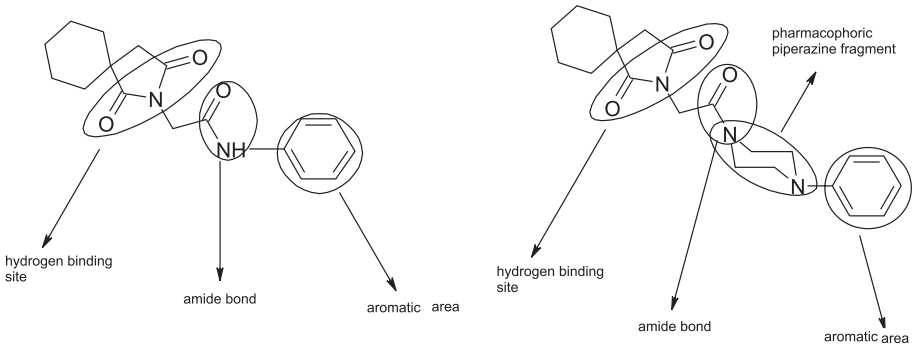
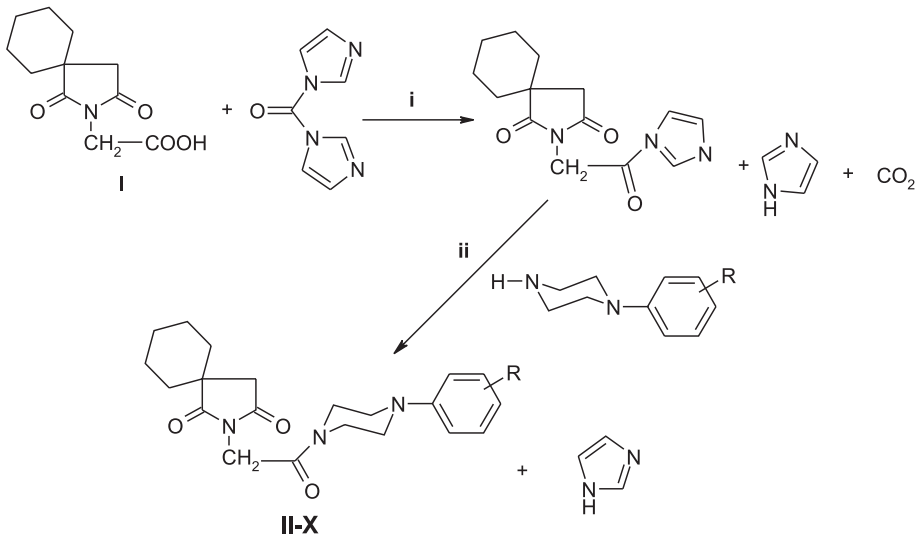


Figure 1. Structural elements and the difference between inactive and active anticonvulsant compounds

Table 1. Physicochemical data for compounds **II-X**

No.	Molecular Formula Mass	Yield% Mp.[^{0}C]	Analyses calc./found			R_f[a]
			%C	%H	%N	
II	$C_{21}H_{27}O_3N_3$ 369.5	69 176-178	68.27/68.5	7.37/7.5	11.37/11.4	0.54A 0.80 B
III	$C_{21}H_{26}O_3N_3F_1$ 387.5	65 136-138	65.10/65.0	6.76/6.9	10.85/10.8	0.64 A 0.85 B
IV	$C_{21}H_{26}O_3N_3Cl_1$ 403.9	62 162-164	62.45/62.2	6.49/6.7	10.40/10.4	0.62 A 0.82 B
V	$C_{21}H_{26}O_3N_3Cl_1$ 403.9	81 145-147	62.45/6.3	6.49/6.8	10.40/10.1	0.58 A 0.85 B
VI	$C_{22}H_{29}O_3N_3$ 383.49	75 150-152	68.90/69.0	7.62/7.7	10.96/10.9	0.56 A 0.84 B
VII	$C_{22}H_{29}O_3N_3$ 383.5	71 167-169	68.90/6.6	7.62/7.9	10.96/10.7	0.62 A 0.86 B
VIII	$C_{22}H_{29}O_4N_3$ 399.5	64 162-164	66.15/65.9	7.32/7.4	10.52/10.5	0.44A 0.80B
IX	$C_{22}H_{29}O_4N_3$ 399.5	68 134-136	66.15/65.9	7.32/7.4	10.52/10.4	0.52A 0.81B
X	$C_{22}H_{26}O_3N_3F_3$ 437.5	63 179-181	60.47/60.2	6.00/6.1	9.62/9.5	0.58 A 0.78 B

[a] Solvents: A – benzene : ethyl acetate: acetone (10 : 5 : 1), B – butanol : acetic acid : water (5 : 4 : 1)



No	II	III	IV	V	VI	VII	VIII	IX	X
R	H	2-F	2-Cl	3-Cl	2-CH_3	3-CH_3	2-OCH_3	3-OCH_3	3-CF_3

i DMF, **ii** room temp., 24 h, cold water.

Scheme 1.

Table 2. ^{1}H NMR spectral data for compounds **II-X**

No.	^{1}H-NMR δ (ppm)/$CDCl_3$
II	1.24-1.93 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.19-3.30 (4H, dt, piperazine, *J* = 5.08 Hz), 3.66-3.81 (4H, dt, piperazine, *J* = 5.08 Hz), 4.36 (2H, s, -CH_2-), 6.94-7.36 (5H, m, arom.)
III	1.23-1.95 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.09-3.19 (4H, dt, piperazine, *J*= 5.02 Hz), 3.67-3.82 (4H, dt, piperazine, *J* = 4.90 Hz), 4.36 (2H, s, -CH_2-), 6.95-7.15 (4H, m, arom.)
IV	1.37-1.95 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.05-3.16 (4H, dt, piperazine, *J* = 4.95 Hz), 3.67-3.83 (4H, dt, piperazine, *J* = 4.95 Hz), 4.36 (2H, s, -CH_2-), 7.03-7.44 (4H, m, arom.)
V	1.40-1.95 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.20-3.31 (4H, dt, piperazine, *J* = 5.08 Hz), 3.65-3.80 (4H, dt, piperazine, *J* = 5.02 Hz), 4.35 (2H, s, -CH_2-), 6.81-7.29 (4H, m, arom.)
VI	1.36-1.96 (10H, m, cyclohexane), 2.36 (3H, s, -CH_3) 2.69 (2H, s, imide), 3.18-3.28 (4H, dt, piperazine, *J* = 5.09 Hz), 3.65-3.80 (4H, dt, piperazine, *J* = 4.90 Hz), 4.36 (2H, s, -CH_2-), 6.76-7.28 (4H, m, arom.)
VII	1.24-1.93 (10H, m, cyclohexane), 2.36 (3H, s, -CH_3), 2.69 (2H, s, imide), 3.18-3.29 (4H, dt, piperazine, *J* = 4.95 Hz), 3.65-3.80 (4H, dt, piperazine, *J* = 4.95 Hz), 4.36 (2H, s, -CH_2-), 6.78-7.29 (4H, m, arom.)
VIII	1.23-1.94 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.06-3.17 (4H, dt, piperazine, *J* = 4.68 Hz), 3.67-3.83 (4H, dt, piperazine, *J* = 4.68 Hz), 3.92 (3H, s, -OCH_3) 4.36 (2H, s, -CH_2-), 6.91-7.11 (4H, m, arom.)
IX	1.23-1.94 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.19-3.30 (4H, dt, piperazine, *J* = 5.09 Hz), 3.65-3.80 (4H, dt, piperazine, *J* = 5.09 Hz), 3.83 (3H, s, -OCH_3) 4.35 (2H, s, -CH_2-), 6.50-7.30 (4H, m, arom.)
X	1.23-1.94 (10H, m, cyclohexane), 2.69 (2H, s, imide), 3.21-3.32 (4H, dt, piperazine, *J* = 5.30 Hz), 3.64-3.79 (4H, dt, piperazine, *J* = 4.68 Hz), 4.32 (2H, s, -CH_2-), 7.07-7.41 (4H, m, arom.)

hexane ring were shown as multiplets within the range of δ 1.24-1.96 ppm. Two protons of pyrrolidine-2,5-dione in all the compounds studied were shown as singlets at δ 2.69 ppm. Protons of the piperazine ring were observed as two dublets of triplets within the range of δ 3.09-3.32 ppm and δ 3.64-3.84 ppm (J = 5.0 Hz). The resonance signal of the methylene spacer between the imide nitrogen and the amide bond occurred as a singlet at δ 4.36 ppm. The signals of aromatic protons appeared as multiplets within the range of δ 6.50-7.41 ppm. The protons of the methyl group [**VI, VII**] were observed as a singlet at δ 2.36 ppm, while three protons of the methoxy group [**VIII, IX**] also occurred as a singlet at δ 3.92 ppm.

EXPERIMENTAL

Chemistry

Melting points (°C) are uncorrected. ^{1}H-NMR spectra were obtained with a Varian Mercury spectrometer working at 300 MHz. Chemical shifts were described as parts per million (δ ppm), $(CH_3)_4Si$ (TMS) was used as an internal standard. Signal multiplicities were given by the following abbreviations: s (singlet), dt (doublet of triplets), m (multiplet). The purity of the compounds was checked by thin-layer chromatography (TLC) performed on Merck silica gel GF_{254} aluminium sheets using the following developing systems: A – benzene : ethyl acetate: acetone (10 : 5 : 1), B – butanol : acetic acid : water (5 : 4 : 1). The spots were detected by means of their absorption under UV light and by visualization with 0.05 mol I_2 in 10% HCl.

GENERAL PROCEDURE FOR PREPARING *N*-4-ARYLPIPERAZIN-1-YL AMIDES OF (2-AZA-1,3-DIOXOSPIRO[4.5]DEC-2-YL) ACETIC ACID [**II-X**]

(2-Aza-1,3-dioxospiro[4.5]dec-2-yl)-acetic acid [**I**] (0.02 mol) was dissolved in 20 ml of DMF, and then *N*,*N*-carbonyldiimidazole (0.02 mol) was added. The mixture was stirred for 0.5 h at room temperature. Afterwards, the appropriate substituted 4-arylpiperazine (0.02 mol) was added. After 24 h of stirring at room temperature, the final reaction mixture was left in an ice-cold bath, and next, the product was precipitated with cold water; it was purified by recrystallization from isopropyl alcohol. Physicochemical data, yields, elemental analyses and R_f values are presented in Table 1. The ^{1}H-NMR spectral data are shown in Table 2.

PHARMACOLOGY

Preliminary pharmacological tests of compounds **II-X** were provided through the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda) by testing procedures which had been described earlier (23, 24). Phase I studies of the investigated compounds involved three tests: a maximum electroshock (MES), a subcutaneous metrazole (sc. Met) and a rotarod test for neurological toxicity (TOX).

In the MES and sc. Met tests, mice are tested for 30 min and 4 h, using the following doses 30, 100 and 300 mg/kg of tested compound. The compound was injected intraperitoneally as a suspension in a 0.5% methylcellulose/water mixture, in a volume of 0.01ml/g body weight. In the MES seizure test an electrical stimulus of duration 0.2 s (50mA) is delivered via corneal electrodes.

In the sc. Met test, a dose of 85 mg/kg metrazole (in mice) was administrated subcutaneously. This produced clonic seizures lasting for a period of at least five seconds in 97 per cent (CD_{97}) of animals tested. Absence of clonic seizure in the observed time of period indicated the ability of compound to abolish the effect of metrazole on seizure threshold.

A neurological toxicity test (TOX) induced by a compound was detected in mice using a standardized rotarod test. Untreated control of mice, when placed on a 6 r.p.m rotation rod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of a mouse to maintain equilibrium for one min. in each of three successive trials. For these experiments, four animals were used at 30 and 300 mg/kg and eight at 100 mg/kg.

The compounds were classified according to the following categories: anticonvulsant activity at 100 mg/kg or less (class 1), anticonvulsant activity at doses higher than 100 mg/kg (class 2), compounds inactive at 300 mg/kg (class 3).

RESULTS

The initial anticonvulsant activity and neurotoxicity data on the compounds in question are presented in Table 3. The MES and sc. Met tests have become the most widely employed seizure models for an early identification and throughout screening of investigational antiepileptic drugs (25). The tested compounds [**III-VI** and **VIII-IX**] at the three doses used (30, 100 and 300 mg/kg) were found to be de-

Table 3. Anticonvulsant screening project (ASP) phase I in mice.

Comp.	Dose	MES[a]		sc.Met[b]		Tox[c]		ASP[d]
	mg/kg	0,5h	4h	0,5h	4h	0,5h	4h	Class.
	30	0/1	0/1	0/1	0/1	0/4	0/2	
II	100	0/1	0/1	2/5	0/1	0/8	0/4	1
	300	0/1	0/1	1/1	0/1	1/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
III	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	0/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
IV	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	1/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
V	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	0/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
VI	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	0/4	0/2	
	30	0/1	0/1	1/5	0/1	0/4	0/2	
VII	100	0/1	0/1	1/1	0/1	0/8	0/4	1
	300	0/1	0/1	1/1	0/1	0/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
VIII	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	1/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
IX	100	0/1	0/1	0/1	0/1	0/8	0/4	3
	300	0/1	0/1	0/1	0/1	1/4	0/2	
	30	0/1	0/1	0/1	0/1	0/4	0/2	
X	100	0/1	0/1	1/1	0/1	0/8	0/4	1
	300	0/1	0/1	1/1	0/1	0/4	0/2	

[a] Maximal electroshock (number of animals protected/number of animals tested); [b] Subcutaneous metrazole test (number of animals protected/number of animals tested); [c] Rotarod toxicity (number of animals exhibiting toxicity/number of animals tested); [d] The ASP classification is as follows: 1 – anticonvulsant activity at doses of 100 mg/kg or lower; 2 – anticonvulsant activity at doses higher than 100 mg/kg; 3 – compound inactive at a dose of 300 mg/kg.

voided of activity in both the MES and the sc. Met tests and showed no neurotoxicity at any of the administrated doses. Compounds **II, VII** and **X** revealed anti-sc. Met activity indicating their ability to elevate the seizure threshold. In that test, the most potent was 3-methylphenylpiperazin-1-yl amide of (2-aza-1,3-dioxo-spiro[4.5]dec-1-yl)-acetic acid **[VII]** and protected animals at doses of 30, 100 and 300 mg/kg after 0.5 h. Compound **II** without substituents at the aryl ring and its 3-CF_3 analogue **X** were also active at doses of 100 and 300 mg/kg in the same test.

In conclusion, as shown in Figure 1, the introduction of a piperazine moiety as an amide fragment in place of phenylamine plays an essential role in inducing anticonvulsant activity. It is noteworthy that also type and position of substituents at the aryl ring are very important for this effect. As has been reported by many studies (26-31), the substitution of the aryl ring with an electron-withdrawing -CF_3 or an electron-donating –CH_3 group was generally beneficial to the biological activity. In the present study we also obtained anticonvulsant active compounds with -CH_3 [**VII**] and -CF_3 [**X**] substituents connected to the aryl ring at position 3. In contrast, introduction of substituents such as chlorines [**III-V**] or methoxy group [**VIII, IX**] led to the loss of anticonvulsant activity in both the tests used.

In the nearest future, on the basis of the hitherto obtained results we intend to synthesize some

new 4-arylpiperazin-1-yl amides of (2-aza-1,3-dioxospiro[4.5]dec-1-yl)-propionic and butyric acid to study the influence of the distance between the imide nitrogen atom and piperazine moiety on the anticonvulsant activity.

Subsequent studies will be published shortly.

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